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DIETARY INTERRELATIONSHIPS — VITAMIN C AND THE METABOLISM OF AROMATIC AMINO ACIDS

By RUTH WOODS

As the science of nutrition advances, it is becoming increasingly apparent that the fundamental dietary essentials—proteins, fats, carbohydrates, vitamins and minerals—can no longer be described solely in terms of their individual respective contributions to the body economy. It is no longer possible to delineate the functions of a particular vitamin in the relatively restricted terms which characterized the discussion of nutrition principles during the early history of this science. Pyridoxine, to cite a typical example, which has been discussed in detail in this publication,* is no longer an "anti-dermatitis factor;" it is part of an enzyme system involved in decarboxylations, transaminations and possibly still other biochemical functions. In order to appreciate the dietary significance of pyridoxine, it is necessary to understand the intricacies of intermediate protein and amino acid metabolism; the nature of enzymatic reactions, etc. Only then, in the light of the metabolic interrelationships in which this factor is involved, does the picture of this vitamin as a nutritive essential come into true focus. The same holds true for all of the other vitamins and dietary factors. A discussion of one involves, eventually, a discussion of almost all of them.

In this way, recent findings have made it elementary to describe vitamin C simply as the antiscorbutic vitamin. With the unfolding of the biochemical action of this vitamin it is now becoming apparent that intimate interrelations exist among ascorbic acid, the proteins and amino acids, the B complex vitamins and certain of the minerals. The present Review will set forth some of the newer findings concerning the role of vitamin C in the body in terms of its effect on the metabolism of certain amino acids (tyrosine and phenylalanine) and the overlapping functions of some of the B vitamins in this aspect of vitamin C behavior.

Vitamin C and Pigment Formation:

The implication of vitamin C in protein metabolism had its origin, somewhat paradoxically, even before the vitamin was identified. During the course

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**cf. Borden's Review of Nutrition Research,* March, 1949, "The Nutritional Role of Pyridoxine," and April, 1949, "Dietary Interrelationships: Pyridoxine and Amino Acid Metabolism."

of studies on cellular oxidations, Szent-Gyorgyi (1) in 1928 isolated a potent reducing agent from cabbage and from adrenal glands. He named the compound "hexuronic acid" because of its close relation to a hexose sugar.

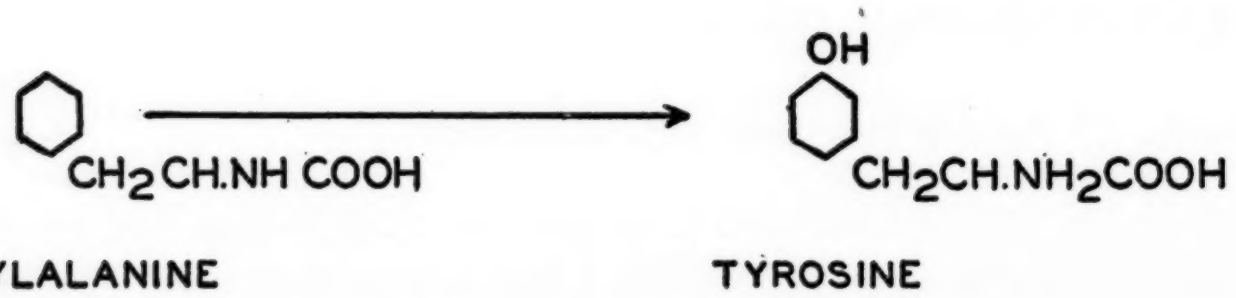
Prior to Szent-Gyorgyi's oxidation studies, investigators in a number of other laboratories were working on an entirely different problem: the isolation of the unidentified antiscorbutic factor in orange and lemon juice. As early as 1925, potent concentrates of this factor had already been obtained by Zilva (2) who also established many of its chemical properties. In 1932, Waugh and King (3) succeeded in isolating this factor. The same year, the identity of vitamin C, the antiscorbutic factor, with Szent-Gyorgyi's "hexuronic acid" from adrenals was reported almost simultaneously by Waugh and King (4) in the United States and Svirbley and Szent-Gyorgyi (5) abroad.

Vitamin C and the Adrenals: As early as 1930, before hexuronic acid had been identified as a vitamin, Szent-Gyorgyi (6) wrote, "Hexuronic acid (isolated from the suprarenal cortex) completely inhibits the formation of pigment in all systems in which a melanoid* pigment is formed through the oxidation of a phenol." He went on further to speculate upon the possible role of this reducing agent in the pathological condition known as Addison's disease in which there is destruction of the adrenals. This disease is often characterized by a peculiar bronzelike pigmentation of the skin, also by anemia, digestive failure and other symptoms; and is usually fatal. He postulated that "the absence of hexuronic acid in Addison's disease could give thus a clear explanation of the mechanism of formation of pigment."

The Mechanism of Pigment Formation: The formation of melanin pigments is a complex phenomenon, the explanation of which is beyond the scope of this Review. To facilitate understanding of the metabolic interrelationships to be discussed, however, some key reactions in this phenomenon are listed briefly below:

(1) The amino acids, phenylalanine and tyrosine are precursors of melanin.

(2) Phenylalanine (an essential amino acid) is oxidized to yield its derivative, *p-hydroxyphenylalanine,* which is tyrosine.



PHENYLALANINE TYROSINE

*Any pigment derived from melanin, the basis of brown and black pigments in humans and animals.

(3) Tyrosine, in one of the many metabolic pathways in which it is involved, is further oxidized to yield 3:4 *dihydroxy-phenylalanine,* commonly abbreviated to *dopa.* Dopa, in turn, is oxidized to the pigment melanin. These reactions are catalyzed by specific enzymes, notably *tyrosinase* and *dopa-oxidase.*

(4) Tyrosine, following another metabolic pathway, also gives rise to the hormone, adrenalin which itself is involved in pigment formation.

(5) Vitamin C, whether by virtue of its strong reducing properties or by means of other mechanisms, inhibits the oxidative processes involved in the conversion of melanin precursors (phenylalanine, tyrosine, dopa) into melanin pigments and thus to some extent, controls the degree of pigmentation in the body. The absence of vitamin C, therefore, might permit excessive pigmentation. This is believed to be a partial explanation of the pigmentation phenomenon characteristic of Addison's disease.

The Role of Vitamin C in Pigment Formation: Many investigators have confirmed the inhibitory influence of vitamin C in pigment formation. Following Szent-Gyorgyi's (5) observation on this action of "hexuronic acid," Abderholden (7) and others (8, 9) demonstrated *in vitro* a similar inhibition of pigment formation from either adrenalin or dopa by vitamin C both with and without the presence of the enzyme, tyrosinase.

Clinical studies in cases of Addison's disease have, in general, tended to further support the role of vitamin C as an inhibitor of pigment formation. A great many investigators (10, 11, 12, 13) have reported a decrease in the deposition of pigment in Addison's disease, or even a definite bleaching effect upon oral or parenteral administration of vitamin C in doses of the order of 500-600 mg. per day.* The bleaching effect has been attributed to direct reduction by vitamin C of melanin to lighter pigments (12).

Vitamin C and Intermediate Protein Metabolism:

The biochemical experiments and the clinical observations in Addison's disease leading to the implication of vitamin C in pigmentation processes, pointed clearly to a role of this vitamin in intermediate protein metabolism. These findings stimulated Sealock and associates (14) at the University of Rochester to a further investigation of the problem.

They fed the melanin precursors, tyrosine and dihydroxyphenylalanine (dopa), to guinea pigs. These animals, unlike the rat, are unable to synthesize vitamin C and are therefore susceptible to vitamin C deficiency. The animals were given daily supplements of the amino acids, both with and without the maintenance level of vitamin C. Signs of vitamin C deficiency became apparent in those animals receiving the amino acid supplements.

*Normal daily requirement is about 75 mg. per day.

This was evidence of an increased requirement for the vitamin when these specific amino acids and their derivatives are being metabolized. Since albino (colorless) animals are incapable of synthesizing melanin, the same experiment was repeated with albino guinea pigs. In these non-pigmented animals, administration of the melanin precursors caused no signs of heightened vitamin C requirement. In other words, these animals do not form pigment, hence do not metabolize tyrosine and dopa for this purpose, and thus apparently present no need for extra vitamin C for this particular purpose.

The Phenomenon of Alkaptonuria: Defects in the metabolism of the melanin precursors, phenylalanine, tyrosine and dopa, lead to a number of derangements in the mechanism of pigment formation. Some of these are manifested in the skin, as for example, the abnormal deposition of pigment in Addison's disease; or conversely, the absence of sufficient pigmentation which occurs in albinism. In addition to the skin manifestations, evidence of deranged metabolism of the aromatic amino acids or their phenolic derivatives may also appear in the form of incomplete products of metabolism in the urine. This phenomenon, termed alkaptonuria, phenylketonuria, or tyrosinosis (depending on the nature of the metabolic intermediates excreted) is characterized by "black urine," i.e., a darkening of the urine on standing due to the presence of abnormal pigment constituents.

The phenomenon of alkaptonuria (as well as the other types of pigmented urine) occurs as a congenital, inborn error of metabolism. The metabolic errors consist of a defect in one or more of the enzyme systems involved in the oxidation of phenylalanine or tyrosine and their various derivatives. The point of blockage due to enzyme defect may occur at any one of the various phases of the intermediate metabolism of the aromatic amino acids (Fig. 1). In alkaptonuria, for example, the defect consists in the specific inability to metabolize homogentisic acid which, in consequence, appears in the urine.

> In *phenylketonuria,* which is characterized by mental deficiency and the excretion of phenylpyruvic acid in the urine, the enzymatic defect results in an inability of the organism to form p-hydroxy analogs from phenylalanine and from phenylpyruvic acid (Fig. 1). The metabolism of tyrosine is unimpaired. In *tyrosinosis,* on the other hand, the defect is in the metabolism of tyrosine and consists of a blockage at the point where p-hydroxyphenylpyruvic acid should normally be converted to 2, 5-dihydrozyphenylpyruvic acid (Fig. 1). Homogentisic acid, the next product in the normal metabolic chain, is metabolized perfectly, as has been shown by feeding the compound to a patient with tyrosinosis.

Two types of alkaptonuria have been recognized: (1) A congenital type resulting from the genetic absence of a specific enzyme which is found in the blood of normal persons and (2) an experimental type resulting from

the feeding of large amounts of phenylalanine, tyrosine or dopa in the absence of adequate amounts of vitamin C.

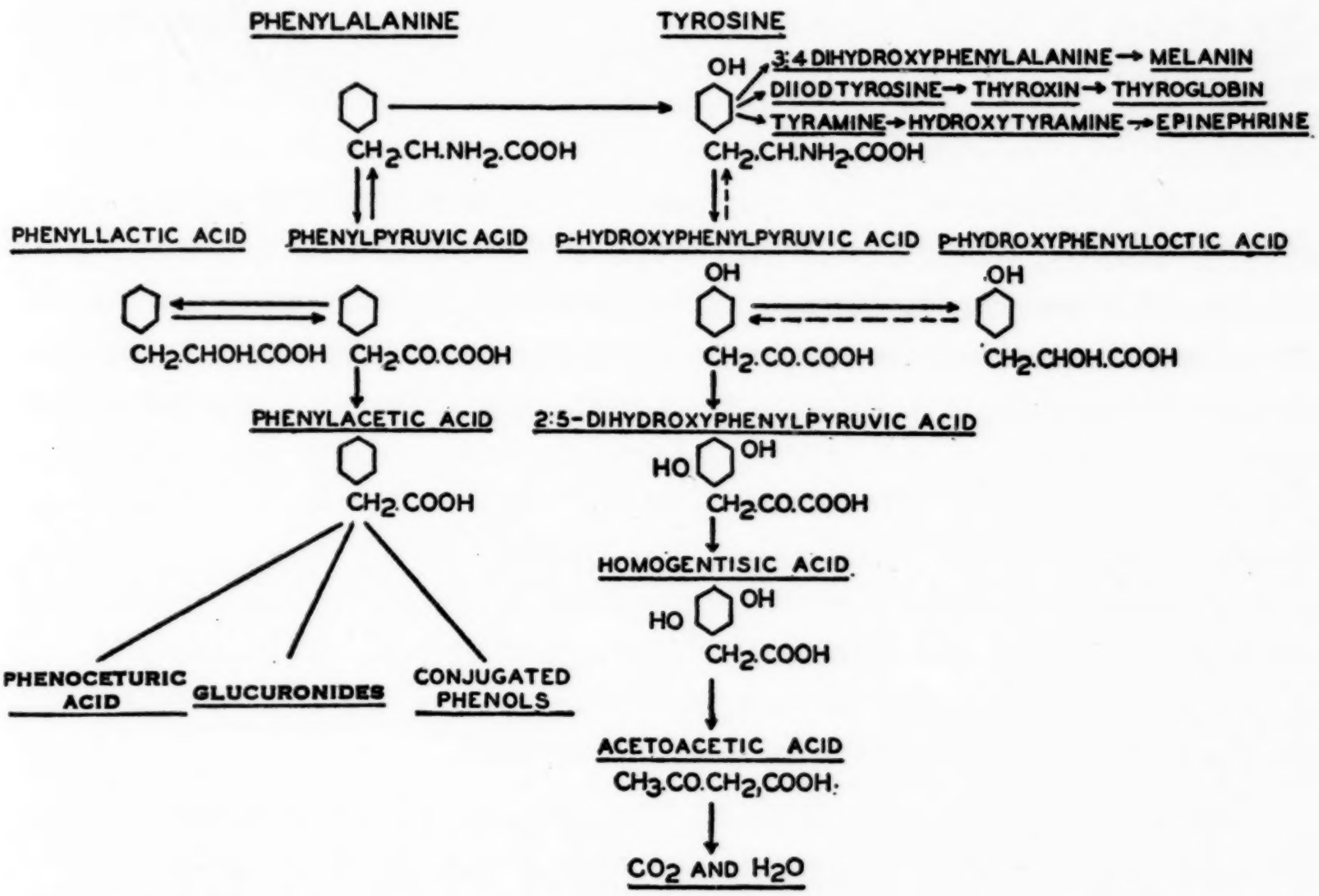


Fig. 1 Intermediary Metabolism of Aromatic Amino Acids* (15)

Experimental Alkaptonuria: In their further investigation of the effect of added tyrosine and dopa on the increased requirement for vitamin C in the guinea pig, Sealock and coworkers (14) discovered another unexpected phenomenon. Animals receiving supplements of these amino acids and derivatives in the presence of only maintenance amounts of ascorbic acid were found to excrete an unidentified pigment-like material in the urine. Analysis revealed it to be homogentisic acid (dihydroxyphenylacetic acid), an intermediate product of tyrosine metabolism (Fig. 1). These investigators realized that they had thus produced an experimental form of the human genetic disorder known as alkaptonuria.

The production of experimental alkaptonuria had been reported the previous year by Papageorge and Lewis (16) and Butts, et al (17) by feeding large amounts of phenylalanine to another species, the white rat. The studies of Papageorge and Lewis were undertaken to determine whether

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*The intermediary metabolism of aromatic amino acids in the human organism has not yet been definitely established. The above diagram represents a simplified version of the generally accepted pathways (15a).

homogentisic acid is a normal intermediate or phenylalanine metabolism or whether it is due to a genetically different metabolic function peculiar only to individuals with congenital alkaptonuria. The production of experimental alkaptonuria by dietary means proved for the first time that homogentisic acid is a step in the normal pathway of phenylalanine metabolism (16, 17) and of tyrosine metabolism (14).

The Role of Vitamin C in Experimental Alkaptonuria: During the course of their studies, Sealock and Silberstein (18) noted that the amount of homogentisic acid excreted seemed to be inversely proportional to the amount of vitamin C received. Investigation of this point led them to the demonstration of the fact that this type of experimental alkaptonuria is dependent upon a deficiency of the vitamin. They showed, further, that when this deficiency is corrected, the intermediate metabolite—homogentisic acid—no longer appears in the urine. Thus, the feeding of 0.5 gram of l-tyrosine with an amount of basal diet containing only 0.5 milligram of ascorbic acid per day resulted in the excretion of homogentisic acid. With the addition of 5 mg. of ascorbic acid per day, all but a trace of the homogentisic acid disappeared from the urine within one or two days.

These experiments were repeated with two normal human subjects (18). On a diet nearly free of vitamin C, the daily ingestion of l-tyrosine resulted in the excretion of significant amounts of homogentisic acid. This could be completely prevented by the ingestion of fairly large doses of ascorbic acid.

Shortly thereafter, the same investigation was carried out on a patient suffering from hereditary alkaptonuria. Sealock and associates (19), as well as other investigators (20, 21), found that in this type of alkaptonuria, the administration of vitamin C is ineffective in preventing the excretion of homogentisic acid and correcting the metabolic defect. These observations led to the conclusion that the metabolism of this compound in the normal individual is probably accomplished by several different enzyme factors. It was suggested that vitamin C is probably not the missing factor in the hereditary condition, but rather some other as yet undetermined factor.

Of final interest in this phenomenon, is the specificity of ascorbic acid action in correcting the defects of aromatic amino acid metabolism. This was demonstrated (22) by the fact that while 10 mg. of ascorbic acid prevent the defect, 10 mg. of d-isoascorbic acid (a structural analog with identical reducing properties but with only insignificant antiscorbutic properties) are ineffective in replacing the vitamin. By providing an amount equivalent to the natural vitamin in antiscorbutic activity, however, it was then possible to prevent the excretion of the abnormal derivatives.

Vitamin C and Amino Acid Metabolism in Infants:

Coincident with the biochemical studies of Sealock and associates, and of other investigators, unexpected confirmation of the importance of vitamin C in the metabolism of phenylalanine and tyrosine was obtained by Levine, Marples and Gordon (23) in the field of pediatrics. These studies, begun in 1936, were concerned with the nitrogen metabolism of premature babies. A falsely positive test for creatine, the compound then under investigation, was traced to the presence of interfering substances in the urine. This chance discovery led to a new series of experiments designed to determine the identity of the unknown substances; the reasons for their occurrence and means of prevention; as well as their physiological and clinical significance.

Studies with Premature Infants: The presence of the unknown compounds was first noted when the diet of premature infants was changed from human milk to cow's milk mixtures. Since cow's milk is twice as rich as human milk in protein content, it was suggested that the unknown compounds might be intermediates of protein metabolism. It was suspected that the specific dietary precursors of these compounds were the amino acids, phenylalanine and tyrosine. This was confirmed by feeding the pure amino acids and, finally, by chemical identification of the urinary compounds as p-hydroxyphenylpyruvic and p-hydroxyphenyllactic acids,* derivatives of tyrosine (Fig. 1). During these and subsequent studies (24) it was noted that although the metabolic defect was exhibited only on diets of higher protein content, its occurrence was somewhat irregular. When the diets were not supplemented with vitamin C the excretion of abnormal urinary compounds occurred consistently; further, addition of vitamin C in adequate amounts completely prevented their excretion.

Full-Term Infants: Full-term infants, with one or two rare exceptions, never exhibited hydroxyphenyluria (excretion of the aforementioned tyrosine derivatives in the urine) even on very high protein diets devoid of vitamin C. Because single doses of tyrosine and phenylalanine always increased the degree of existing hydroxyphenyluria in premature infants, however, it was decided to examine the effect of these amino acids on full-term babies.

The capacity of even full-term infants to handle these amino acids contained in their customary feedings of cow's milk proved to be limited. Their tolerance could be exceeded by supplements of the pure amino acids in the absence of vitamin C. The feeding of a single dose of 1.0 gram of tyrosine

*Also found by Sealock and Silberstein (22), in addition to homogentisic acid, in experimental alkaptonuria (cf. page 11).

or phenylalanine per kg. of body weight was apparently sufficient to disrupt the enzymatic mechanisms responsible for complete oxidation of these amino acids, *whereas the daily ingestion of slowly absorbed, divided doses of one-half this amount (0.5 gm. per kg. as components of the protein of the basal cow's milk diet [25]) did not flood the cells to the point of precipitating the defect in full-term infants as it did in premature subjects.*

Vitamin C corrected both the artificially induced defect in full-term babies fed pure amino acids and the spontaneous defect in premature babies fed cow's milk rich in protein. Further, the administration of Vitamin C (from 70 to 450 mg.) to full-term infants prior to dosage with the pure amino acids prevented the artificial induction of hydroxyphenyluria.

These findings pointed to an interesting interaction among three factors: The level of protein intake, the degree of vitamin C saturation of the tissues, and the maturity of the infant. Whether the feedings consisted of cow's milk or evaporated skimmed human milk, both richer in protein than ordinary human milk, the absence of vitamin C in these formulas invariably precipitated the appearance of tyrosine in the urine. The excretion of these derivatives was further heightened by the feeding of single doses of the pure amino acids, tyrosine and phenylalanine.

Specificity of Vitamin C: The specificity of vitamin C in the metabolism of the aromatic amino acids is indicated by several facts:

(1) Isoascorbic acid, an analog of the vitamin with similar reducing properties but only half the antiscorbutic potency, was only transiently effective in large dosage in one infant (15) and in similar large dosage in vitamin C-deficient guinea pigs (22).

(2) Members of the vitamin B complex, vitamins A, D and E, ryzamin (rice) and yeast extract, cortate and creatine given singly and in combination for prolonged periods produced negligible effects in remedying the metabolic disturbance.

(3) In one instance, however, a crude, vitamin C-free liver extract, produced a prompt and persistent eradication of artificially induced hydroxyphenyluria in one full-term infant. It was, however, given with no or slight success to four premature infants (24) and to vitamin C-deficient guinea pigs (26). This led to the suggestion (24) that full fetal maturity may bring with it—in addition to an adequate store of vitamin C—a factor or factors, possibly of hepatic origin, and not solely dependent on vitamin C, capable of assisting in the breakdown of aromatic amino acids.

Mechanism of Vitamin C Action in the Metabolism of Aromatic Amino Acids:

Tyrosine System: One way of facilitating the search for the metabolic

system responsible for the ascorbic acid effect would be to demonstrate that the vitamin action resides in or is more prominent in a single tissue. This objective was, in part, achieved by Lan and Sealock (27). They found that the ability of surviving slices of liver from guinea pigs to oxidize the amino acid, tyrosine, is dependent upon a normal intake of ascorbic acid. Liver from vitamin C-deficient animals failed to exhibit this metabolic activity. The scorbutic liver, however, was shown to regain its ability to oxidize tyrosine upon the addition of vitamin C in both *in vivo* and *in vitro* experiments. They conclude:

> "With the finding that guinea pig liver slices oxidize tyrosine only in the presence of an adequate amount of ascorbic acid, it would appear that the vitamin is a necessary and key component of the enzyme systems responsible for the oxidation of the amino acid. It may even be the prosthetic group of one of the enzymes acting as a respiratory catalyst in tyrosine metabolism."

Lan and Sealock go on, further, to indicate, however, that this hypothesis has not been conclusively demonstrated and point out the possibility of an indirect action of the vitamin as the determining factor.

Dopa System: Although early tyrosine studies indicated the heightened requirement for vitamin C for purposes of melanin formation, later studies showed that the vitamin is required for other metabolic pathways of tyrosine as well. This increased metabolic requirement for vitamin C was even found with albino guinea pigs who are unable to form melanin and do not need it for this one purpose. Studies with the melanin precursor, dopa,—particularly in albino animals—likewise indicated that dopa in its metabolism bears a more complex relationship to vitamin C than that of melanin formation alone. Like tyrosine, the oxidation of dopa was found to occur only in the presence of vitamin C; this time chiefly in kidney rather than liver tissue (28). Similar speculation as to the direct enzymatic role of vitamin C, or some indirect activity, arises in these as in the tyrosine studies. The indication for two separate enzyme systems seems to be strongly suggested.

The interesting suggestion (29) that inadequate oxidation of dopa in the kidney gives rise to pressor amines raises the incidental question as to the role of Vitamin C in the development of hypertension by virtue of the increased dopa concentration resulting in the kidney from a deficiency of the vitamin (28).

Acid-Base Balance: During studies on the effect of feeding other amino acids to vitamin C-deficient animals, it was found that prior feeding with the amino acid, glutamic acid, prevented the excretion of tyrosine intermediates when the latter was fed. The preventive effect, however, was observed only with the first dose of glutamic acid; repeated doses had no

effect. By the use of other dicarboxylic acids or of ammonium chloride (an acidifying agent) the effect of glutamic acid was shown to be related to its acidifying action. Further, inclusion of sodium bicarbonate in the diet inhibits the action of vitamin C supplements in the metabolism of tyrosine. These findings point to the importance of a proper acid-base balance to provide a favorable medium for the action of vitamin C and may also indicate the importance of the acid factor in the direct action of the vitamin.

The Overlapping Function of Liver Factors: A number of observations have led to the discovery that certain liver factors play an important part in the vitamin C-aromatic amino acid interrelation. Thus, patients with untreated pernicious anemia are known to excrete phenolic derivatives in the urine (31) and the oxidation of tyrosine by liver slices from rats deficient in folic acid has been enhanced by addition of this vitamin to the medium (32). Similarly, Levine (15) found one case of response to liver extract by a full-term infant with artificially induced hydroxyphenyluria (see p.....). These observations led Woodruff and Darby (33) to test the effect of folic acid (pteroylglutamic acid) on tyrosine metabolism in the scorbutic guinea pig. They found that folic acid exerts an action similar to that of ascorbic acid in correcting the metabolic defect resulting from tyrosine feeding, since scorbutic animals responded to separate supplements of either folic acid or vitamin C. Shortly thereafter, Sealock and Lepan (34) reported a similar action for anti-pernicious anemia liver extracts. They found, however, that these crude extracts do not increase the *in vitro* oxidation of tyrosine by liver slices as do ascorbic acid and folic acid. Whether liver factors other than folic acid, such as vitamin B_{12}, for example, are involved in this mechanism is still a matter of investigation, as are the effects of folic acid inhibitors (35) and related problems. These findings show the ever-widening interlacings of functions among the various nutritional essentials. In the present story, for example, vitamin C is intimately linked with amino acid metabolism and, through this medium, with folic acid metabolism and, ultimately, perhaps with the action of other B vitamins and enzyme systems.

It can thus be visualized how a disturbance in a remote system involving one nutrient can have repercussions in an apparently unrelated system not immediately concerned with the particular nutrient in question.

Practical Implications:

The clinical significance of the occurrence of a defect in the metabolism of aromatic amino acids in the absence of vitamin C is still conjectural. As stated by Levine (15):

"Even those infants who exhibited the defect over prolonged periods con-

tinued to thrive and showed no overt signs of either amino acid or vitamin C deficiency. They gained weight well, were in positive nitrogen balance and signs of scurvy were absent. Despite the negative evidence, the observations give rise to a number of intriguing clinical questions."

Prominent among the questions raised were the possible relation of the metabolic defect and the morphological changes of vitamin C deficiency, namely improper formation of collagen and intercellular substance. Also the significance of the delicate transparency of skin in premature babies in terms of improper melanin formation. Again, the possible relation between instability of body temperature in premature infants and the production of adrenalin and thyroxine, both ultimately derived from tyrosine. Finally, a theoretical explanation for the commonly postulated role of vitamin C in the development of rickets in terms of the heightened excretion of aromatic organic acids.

Levine has concluded:

"From the practical standpoint and despite the absence of overt signs of deficiency, the observations emphasize the importance of early and adequate administration of vitamin C to all premature infants receiving cow's milk of high protein content."

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